

Research with Biorepositories: Blast Exposure and Neural Damage Lessons from 30+ Years of Experience

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The opinions expressed herein are those of the presenter and are not necessarily representative of those of the government of the United States, the Uniformed Services University of the Health Sciences (USUHS), the Department of Defense (DoD); or, the United States Army, Navy or Air Force.

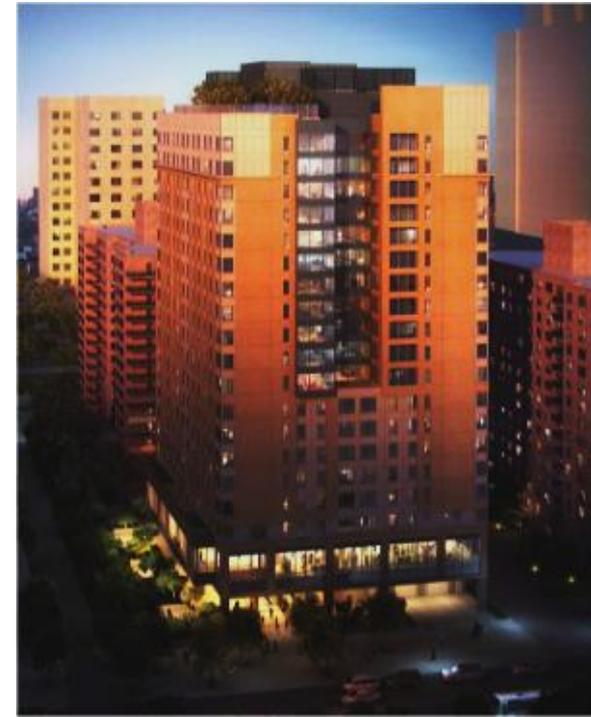
I have no conflicts to report.



Context:

- “The RAC is charged with advising the Secretary of Veterans Affairs (VA) on research related to **understanding and treating the health conditions of Gulf War veterans**. This cohort suffers from **a range of complex, chronic conditions with poorly understood etiologies**. Major areas of emphasis for this meeting will be discussing **how VA can best approach its research strategy on these issues**, particularly related to genetics and genomics studies, and considering ways to better integrate research with clinical care.”

- 1986, recruited to Mount Sinai School of Medicine and served as its Director of Neuropathology for 24 years
- Mount Sinai had just been funded as one of the first 5 NIH/NIA-supported **Alzheimer's Disease Research Center**. Organized and ran the Neuropathology-Brain Bank Core within the Center.
- Also received funding for P01 on "**Normal**" Aging and Early **AD (MCI)**. Ran the Brain Bank Core within the Jewish Home for the Aged (JHA).
 - JHA – 1600 residents in 2 campuses
 - Mean age on admission ~80 years, many entering with normal cognition
 - Mean age at death 83.6 years
 - Academic nursing home - full affiliation with the Mount Sinai School of Medicine
 - Overwhelming % of hospital admissions were to the Mount Sinai Hospital



- 1988, organized P01 to study **ALS/Parkinsonism-Dementia Complex of Guam**, including a Neuropathology/Brain Bank Core. Over 350 brain specimens were collected and sent 8,000 miles from Guam to New York.
- Mid-1990's, helped develop **schizophrenia** Brain Bank at the Pilgrim Psychiatric Center on Long Island (300 brains).
- With Joe Erwin and Patrick Hof, developed the “**Great Ape Brain Bank**”, consisting of chimpanzee, gorilla, orangutan and bonobo brain specimens . Especially in the chimpanzee, a full aging series was accumulated (80+ specimens).



- These 5 brain banks were very successful, distributing thousands of samples to hundreds of researchers throughout the world.
- Based on research studies using these tissue samples, hundreds (perhaps thousands) of peer-reviewed papers were written.

Basic Principle: The value of a brain bank is judged by the number of samples it distributes and how many publications they generate! The better characterized the specimens (and the patients they are derived from), the more useful they are for research.

Alzheimer's Disease:

- Patients do not come with only one disease. Overlapping and mixed brain pathologies common among the elderly
- Our restricted “clean series” (just AD, no other comorbidities) representing all clinical stages of Alzheimer's disease (CDR=0 through CDR=5/6) showed:
 - The relative importance of tau vs beta-amyloid pathology
 - The cholinergic deficit associated with Alzheimer's disease is a relatively late phenomenon (not seen until CDR=3)



Schizophrenia:

- Elderly schizophrenics tend to become demented, often to a severe degree. The neuropathology of that dementia is not based on plaques and tangles.
- Patients with schizophrenia have a decreased density of oligodendroglial cells in certain brain regions (the white matter theory).



ALS/Parkinsonism-Dementia Complex of Guam

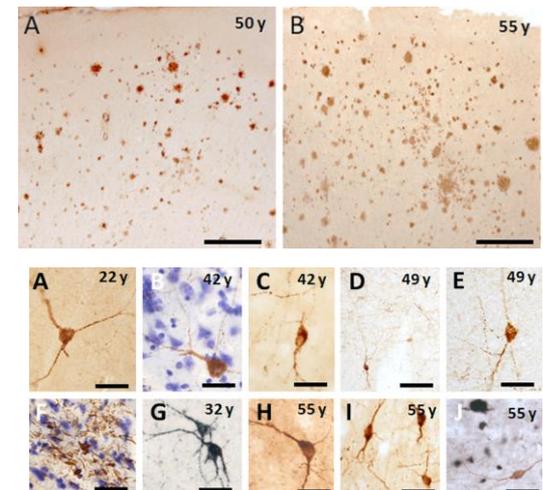
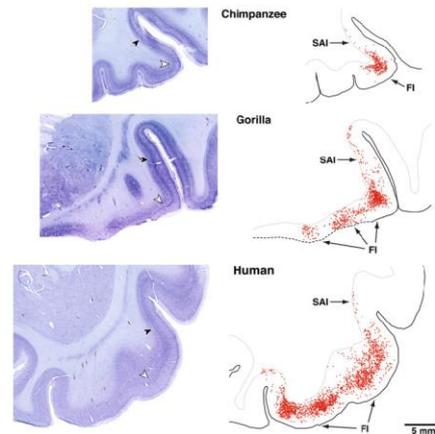
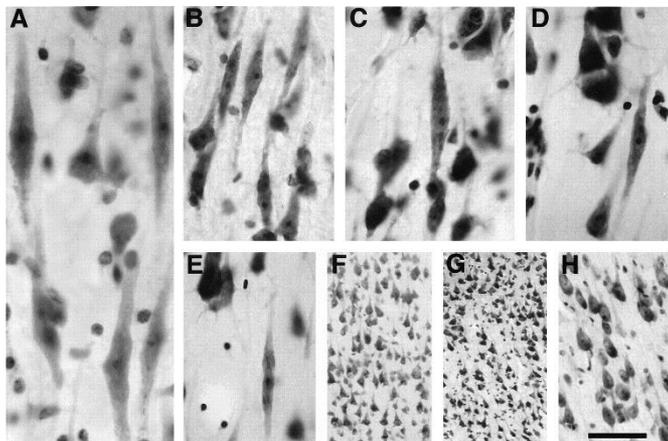
- Characterized the nature of the pathology of ALS-Parkinsonism-dementia complex for the first time using immunohistochemistry and molecular biology approaches.

Stereology

- Showed that stereologic approaches were feasible and could be used as a valuable tool for quantifying neuronal loss and lesion burden in human banked brain specimens.

Great Ape Brain Bank:

- We characterized the von Economo neurons in humans and in great apes.
- The brains of aged gorillas show a significant amount of beta-amyloid accumulation (vascular and A β plaques) and some tau-like neuronal lesions.



Regulatory Issues:

- From the beginning, the **Mount Sinai IRB** stated that since our brain bank activities dealt with deceased individuals, we were, by definition, not engaging in “human research.” Accordingly, they refused to review any of our protocols or procedures.
- Consent forms (really, permission to donate the brain for research by the next-of-kin) were simple and straightforward. The enactment of HIPAA changed things.



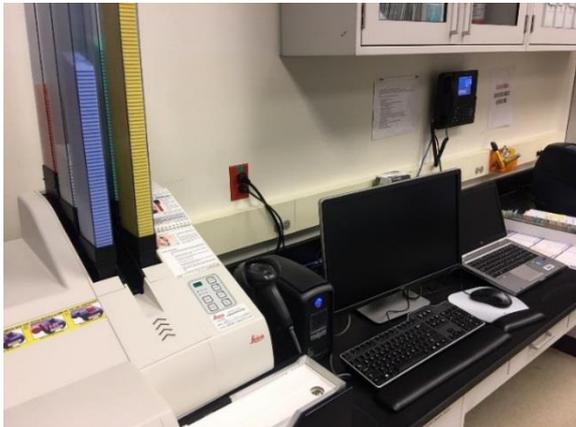
Procedural Issues:

- We were able to obtain specimens relatively rapidly (mean pmi, < 6 hours), making them very useful for research. Attempts to obtain immediate autopsies (i.e., within minutes) did not add much value to the research studies we were supporting. Specimens with lengthy post-mortem intervals (>24 hours) could also be useful. Specimens with prolonged post-mortem intervals (>72 hours) could be used, but only for limited morphology studies (e.g. confirming clinical diagnosis).
- Agonal interval prior to death is an especially important factor for obtaining good DNA/RNA.
- These conditions need to be carefully documented.



Careful and detailed neuropathology work-ups are critical.

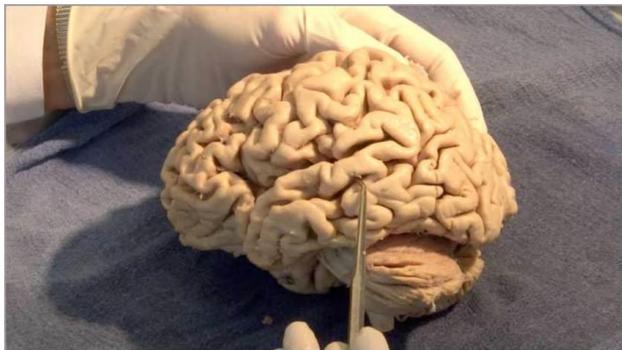
- You cannot take the clinical diagnosis and assume that this is what the patient actually suffered from and also that additional diagnoses were not present. Mistaken diagnoses, multiple diagnoses and important confounding conditions (especially related to disease states leading to the eventual death of the patient) are critical to identify and characterize in all cases. These issues can markedly influence the research data obtained.
- Just checking for the presence/extent of plaques and tangles in a few blocks is not a sufficient workup.
- A robust specimen inventory system is essential!



Dissection Protocols

All brain bank dissection protocols are compromises!

- Obtaining fixed material vs frozen specimens is often in conflict, especially for relatively small neuroanatomic targets. Finding such targets in frozen slabs of brain tissue can be difficult – neuroanatomic expertise is an essential competency. Photograph everything. Using thawed frozen tissues that are then fixed does not produce equivalent results as fixed tissues.
- One can allow for quantification using stereologic approaches, but this must be carefully thought out in advance.



Control Brains

- Availability of properly matched control brain specimens is critical
- Such control specimens are difficult to identify and collect
- Lack of a defined neurologic disorder in the life of the individual does not necessarily mean that the brain is “normal” or that it can appropriately serve as a control
- Remember, control cases have to die in order to become part of your repository. Why the control has died and what has happened to them in that process can be important to whether that specimen should serve as a control for your experiment

IATA* Regulations and Running a Brain Bank

- Formalin is now considered a Hazardous Liquid. You can no longer send shipments containing large quantities of formalin (>30 ml/container).
- The shipping container must have enough specialized absorbent material sufficient to handle the quantity of formalin being shipped.
- Fines for improperly shipped hazardous materials can range from \$250 to \$500,00 per violation (and can include jail time).
- “FedEx Ground Package Systems Inc., therefore, cannot assume any responsibility for omissions, errors, misprinting, or ambiguity contained within this guide and shall not be held liable in any degree for any loss or injury caused by such omission or error presented in this publication.”

* International Air Transport Association



Late 2010, recruited to USUHS and became an employee of the Department of Defense....

Primary Mission: Develop a Brain Bank designed to collect human brain specimens to be used to characterize military traumatic brain injury and its consequences, and to support research on these issues.

- Unique opportunity to build a completely new brain repository facility. How much \$\$\$? space? What kind of equipment? layout? etc, etc.
- Core personnel hired and trained. Training certification for participation in research, especially involving human subjects (even though they were dead) was more extensive than I was used to in the private sector.
- Virtually all brain donations were to be obtained off-site so a variety of logistical details had to be determined, anticipated and dealt with.

The IRB

- The USUHS IRB had never previously dealt with the donation of tissues for use in research (especially from dead people).
- Service members are in a “command condition”, that is, they must obey orders from superiors. Therefore, to some degree, they are a vulnerable population when considered as research subjects.
- Initially, many members of the IRB were wary of the concept of the DoD running a Brain Bank and what their oversight role should be. With time, they realized that a specialized brain bank was essential in order to begin to characterize military TBI and its consequences, the major medical problem facing the DoD. Under the IRB’s guidance, acceptable protocols began to emerge.



- State-of-the-art facility has been built and is functioning with fully approved protocols, consent documents, etc.
- To date, we have collected a total of 48 brain specimens. Of these, 83% served in the military, most with battlefield experience, several with significant blast exposure.
- Of the few non-military (civilian) specimens in the Repository, a small number are from contact sports athletes and from civilians with a history of a single significant episode of head trauma in the past.
- The pace of brain donation is now increasing, especially from at-risk military populations. We are beginning to approach participants in longitudinal study cohorts and are allowing them to sign up as brain donors.

Primary mission - to characterize the long-term effects of blast exposure on the human brain

- In 1916, Dr. Frederick Mott suggested that exposure to blast (in the trenches of World War I) might cause direct damage to the brain and represent an underlying cause of “shell shock.” Mott’s hypothesis remained untested for 100 years.
- “Shell shock”, much like “Gulf War Syndrome”, was a somewhat vague, incompletely characterized disorder, mostly defined by a series of clinical observations.
- My mission was to test this concept, using modern tools of neuropathology to examine brain specimens derived from deceased Service members who had been exposed to blast TBI and suffered significant persistent behavioral-neurologic symptoms.



THE LANCET, FEBRUARY 12, 1916

The Lettsomian Lectures
OR
THE EFFECTS OF HIGH EXPLOSIVES UPON
THE CENTRAL NERVOUS SYSTEM.

Delivered before the Medical Society of London
By FRED. W. MOTT, M.D. LOND., F.R.C.P.
LOND., HON. LL.D. EDIN., F.R.S.,
MAJOR R.A.M.C. (R.), THE LONDON GENERAL HOSPITAL; PATHOLOGIST TO
THE LONDON COUNTY COUNCIL ASYLUMS.

LECTURE I
(Delivered on Feb. 7th.)
MR. PRESIDENT AND GENTLEMEN.—Permit me to
thank you for the great honour the Medical Society

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Common Persistent Symptoms In Post-Blast TBI Subjects

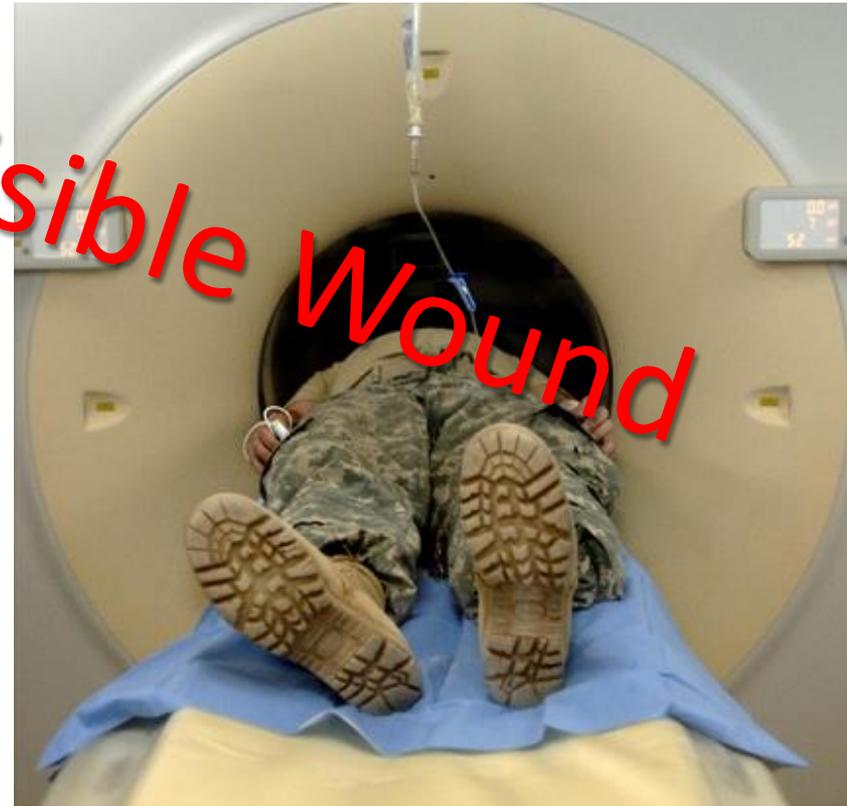
Physical: headache, nausea, vomiting, dizziness, fatigue, blurred vision, sleep disturbance, sensitivity to light/noise, balance problems, hearing difficulties/loss, seizure

Cognitive: impaired attention, concentration, recent memory, speed of processing, judgment, executive function

Behavioral/emotional: depression, anxiety, agitation, irritability, impulsivity, aggression

Can Neuroimaging Studies of Post-Blast TBI Patients Provide Answers?

To date, no routine neuroimaging studies have provided a consistent signal alteration to indicate the presence of pathologic lesions in the brains of post-blast TBI patients with significant persistent symptomatology.



Characterisation of interface astroglial scarring in the human brain after blast exposure: a post-mortem case series



Sharon Baughman Shively*, Iren Horkayne-Szakaly*, Robert V Jones, James P Kelly, Regina C Armstrong, Daniel P Perl

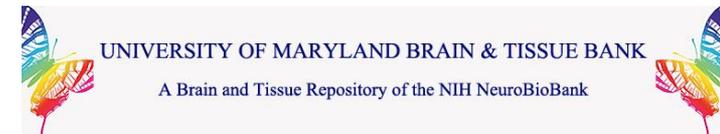
Summary

Background No evidence-based guidelines are available for the definitive diagnosis or directed treatment of most blast-associated traumatic brain injuries, partly because the underlying pathology is unknown. Moreover, few neuropathological studies have addressed whether blast exposure produces unique lesions in the human brain, and if those lesions are comparable with impact-induced traumatic brain injury. We aimed to test the hypothesis that blast exposure produces unique patterns of damage, differing from that associated with impact-induced, non-blast traumatic brain injuries.

Lancet Neurol 2016

Published Online
June 9, 2016
[http://dx.doi.org/10.1016/S1474-4422\(16\)30057-6](http://dx.doi.org/10.1016/S1474-4422(16)30057-6)
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[http://dx.doi.org/10.1016/S1474-4422\(16\)30058-8](http://dx.doi.org/10.1016/S1474-4422(16)30058-8)

Lancet Neurology 15: 944-953, 2016



Long-term Effects of Exposure to Multiple Blasts (Index Case)

- The patient is a former Special Operations combatant who died at age 45 of suicide. The patient served in Desert Storm, OIF and OEF where he was considered to be highly competent, reliable and emotionally stable.
- In combat and training exercises, he had been exposed to hundreds of blasts at close proximity. The effects of these episodes were not disclosed to his superiors for fear of being deemed unfit for duty.
- Once separated from the military he complained of persistent headaches, sleep disturbance, memory problems, and trouble maintaining mental focus.
- The patient had wrestled and boxed in his school years, and had experienced 3 MVAs.

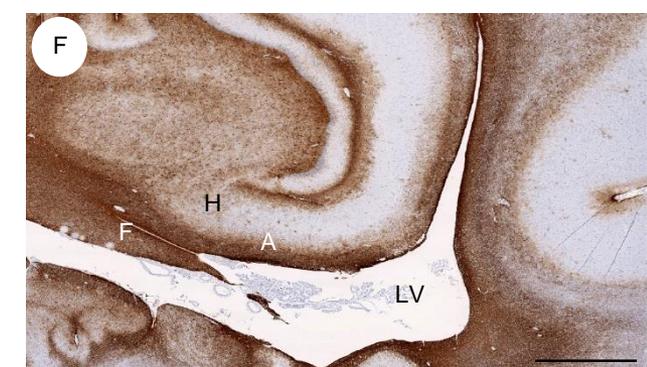
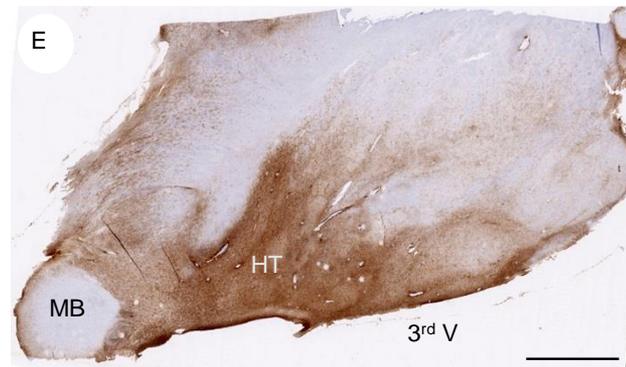
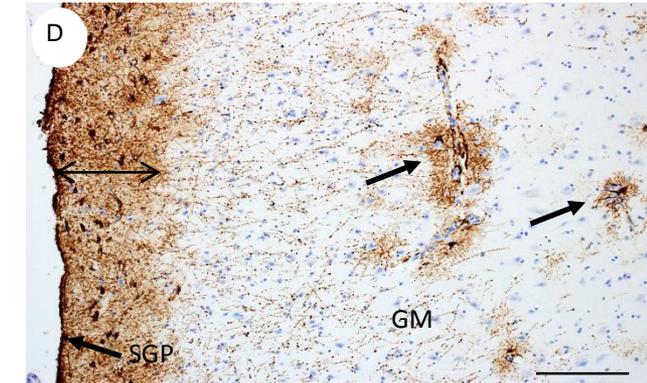
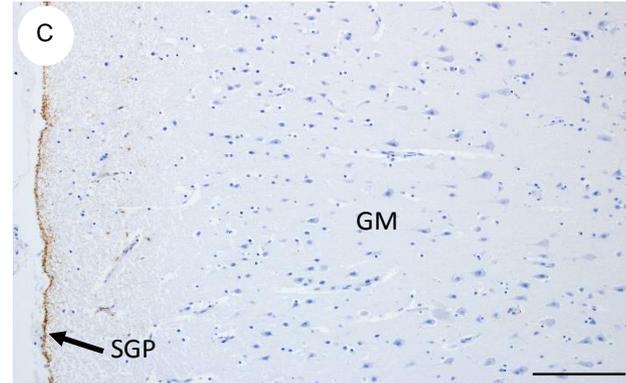
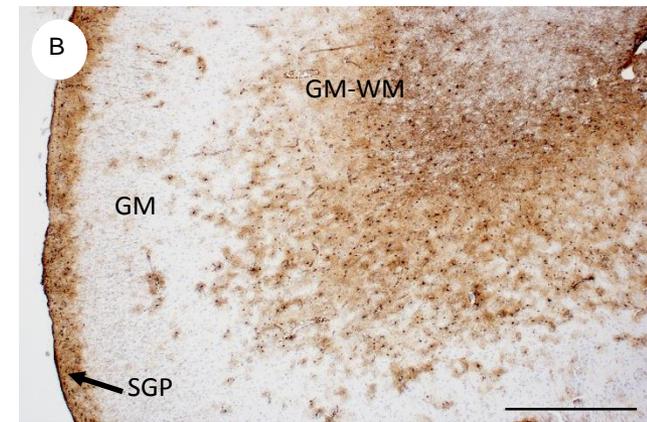
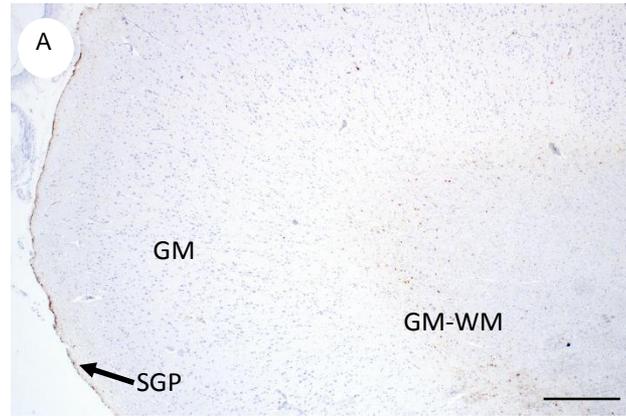
Long-term Effects of Exposure to Multiple Blasts (Index Case)

- He often lost coherence of thought and jumbled speech.
- His wife confirmed short-term memory problems and other cognitive and behavioral changes.
- Following discharge, clinicians described poor eye contact, flat affect and low voice tone. He was diagnosed and treated for PTSD, depression and anxiety.
- 1.5 T MRI, one month prior to death, showed no abnormalities
- There was no indication of substance abuse by history or postmortem toxicology screening.

Interface Astroglial Scarring – GFAP Staining

1. Sub-pial plate (SGP)
2. Cortical penetrating blood vessels
3. Gray-white matter junction (GM-WM)
4. Periventricular structures

This pattern of damage adheres to basic principles of blast biophysics (interface of differing densities). It also correlates with many of the symptoms noted during life (memory problems – scarred hippocampus; sleep disorders – scarred hypothalamus).



A, C – Civilian Impact TBI; B,D,E,F – Chronic blast TBI (Case 1)

Neuropathology of Blast TBI

USUHS/CNRM/JPC/U Maryland Collaboration

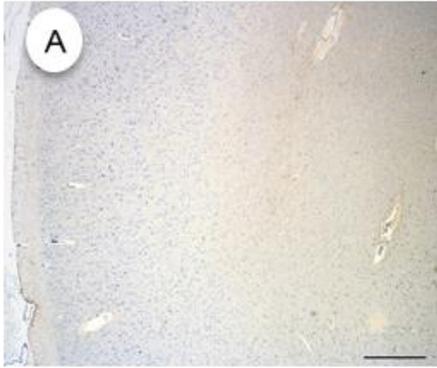


Joint Pathology Center (JPC) Tissue Repository

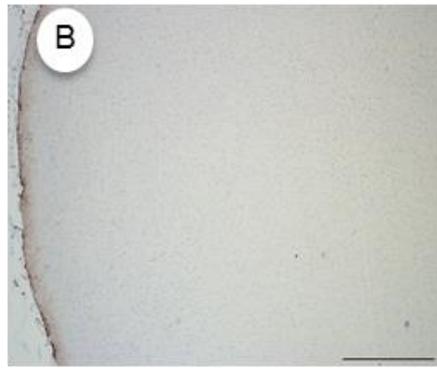


- Collecting tissue samples since 1917
- Repository collection contains:
 - 7.4 million cases
 - 31 million paraffin blocks
 - 55 million glass slides
- Consents for the use of these specimens for research are not available for any of these cases.
- The JPC currently emphasizes its role in providing diagnostic evaluations and has few resources to support research
- They were able to provide access to paraffin blocks from 3 chronic blast TBI cases (ie, survival >6 months) and 3 acute cases (ie, survival < 2 months)

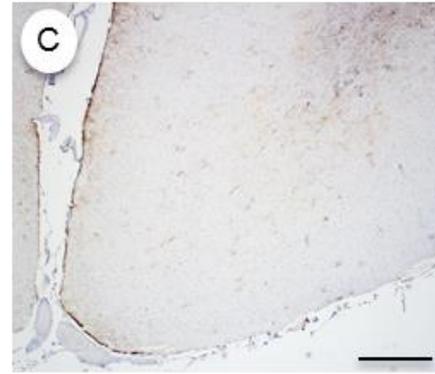
**Chronic Impact TBI
No Blast**



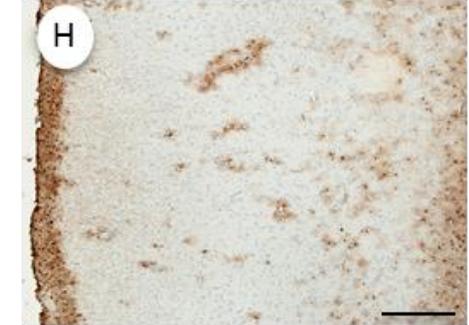
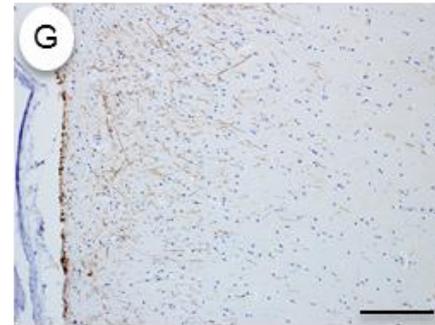
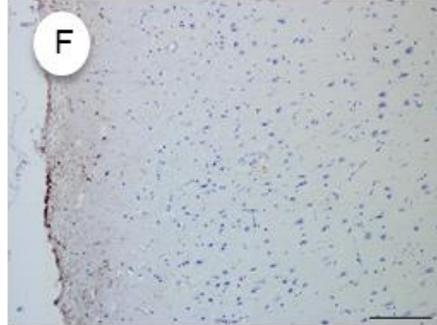
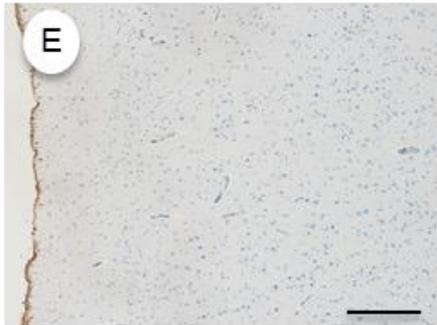
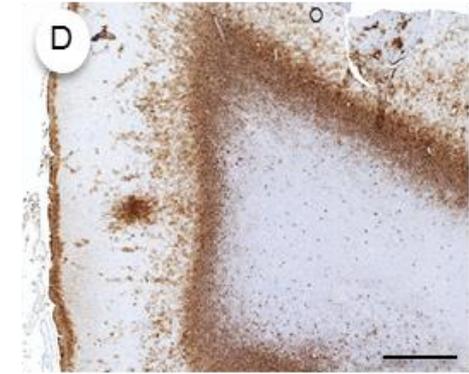
**Chronic Substance
Abuse**



**Acute Blast TBI
4 Day Survival**



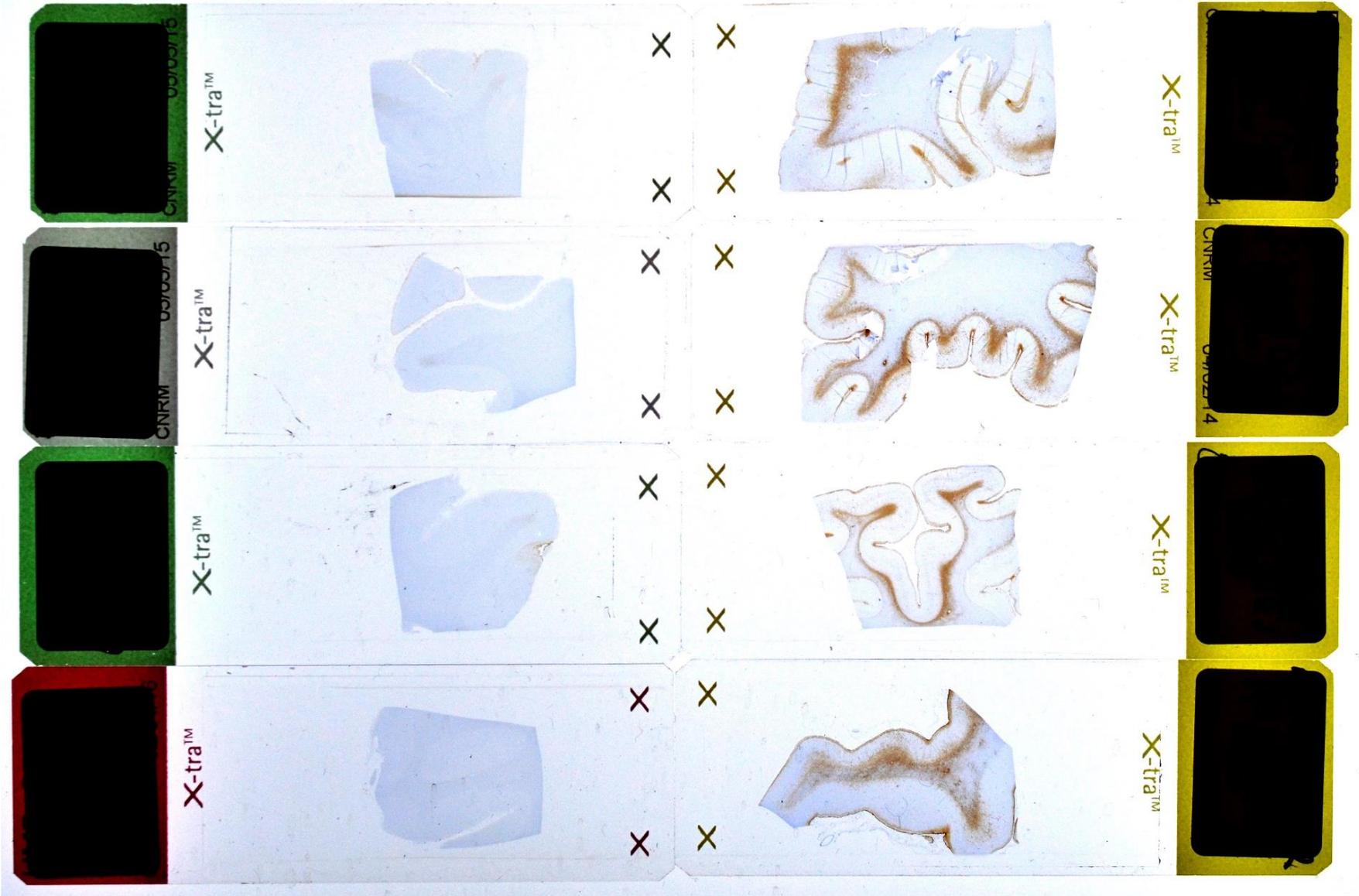
**Chronic Blast TBI
> 6 Month Survival**



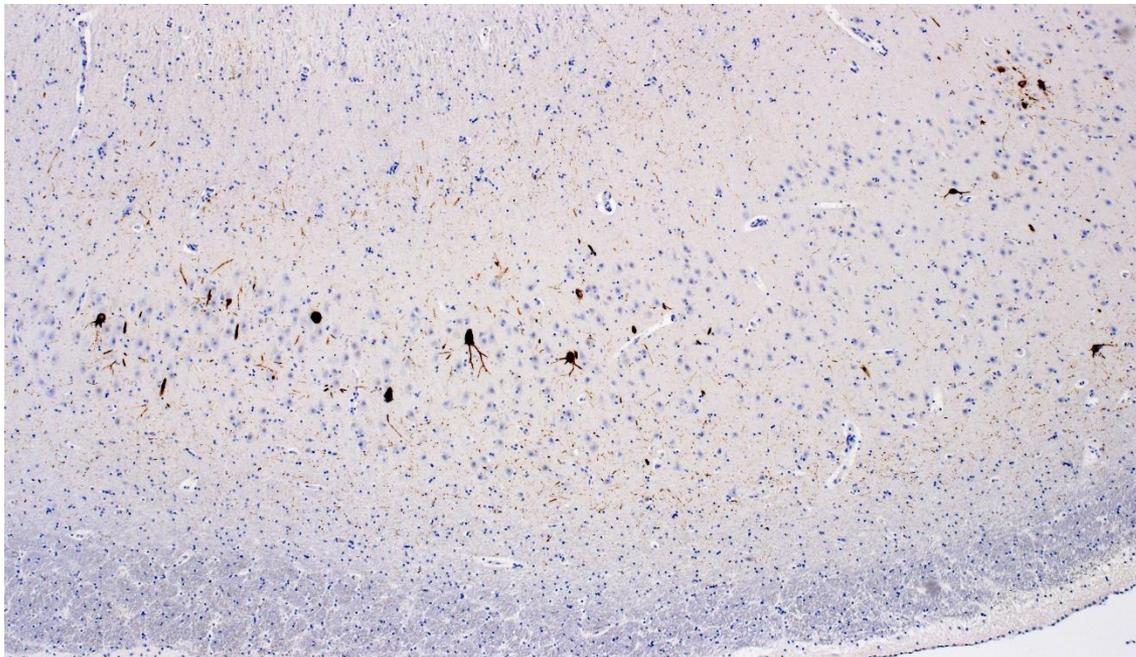
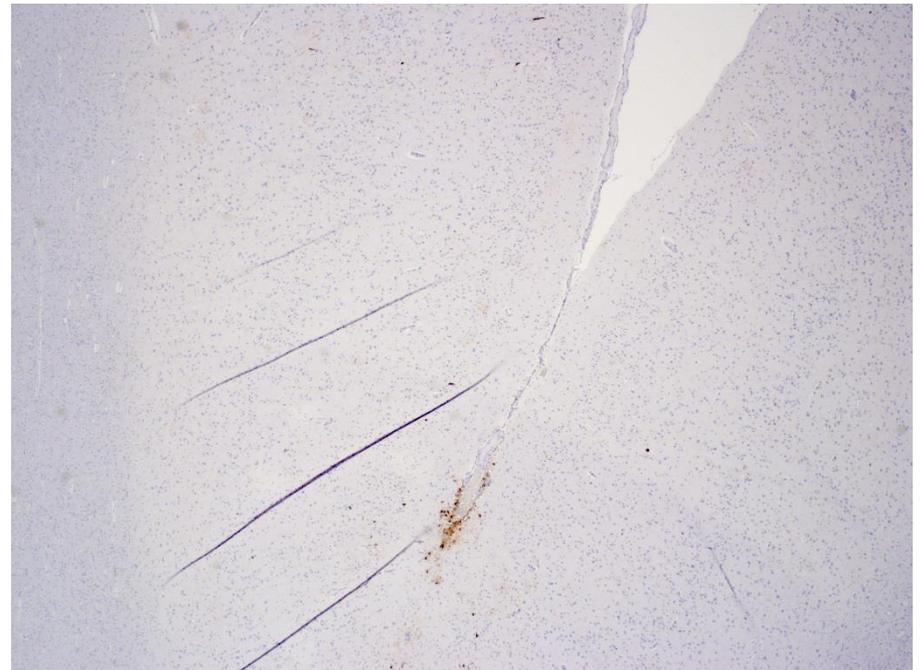
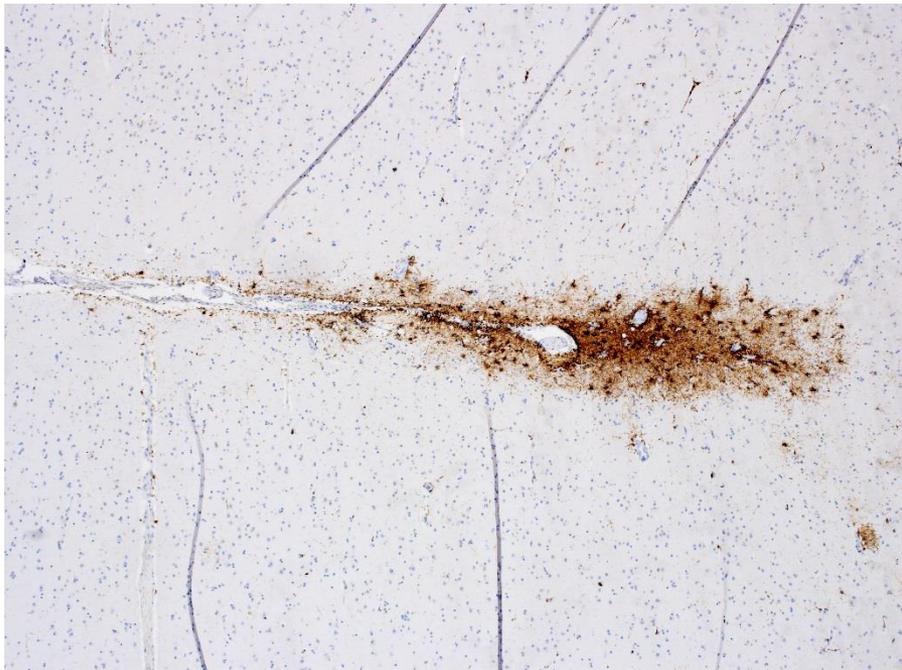
- All 5 chronic blast cases were clinically diagnosed with PTSD
- All showed virtually identical interface astroglial scarring, that is, scarring repair at sites with differing densities (blood/brain, CSF/brain, gray/white matter). This interface pattern fits known principles of blast physics.
- 3 blast cases dying within a few days of the blast event showed the very early features of scar formation in the same locations
- Changes were not seen in cases of impact TBI, substance abuse or CTE.

Impact TBI (no blast)

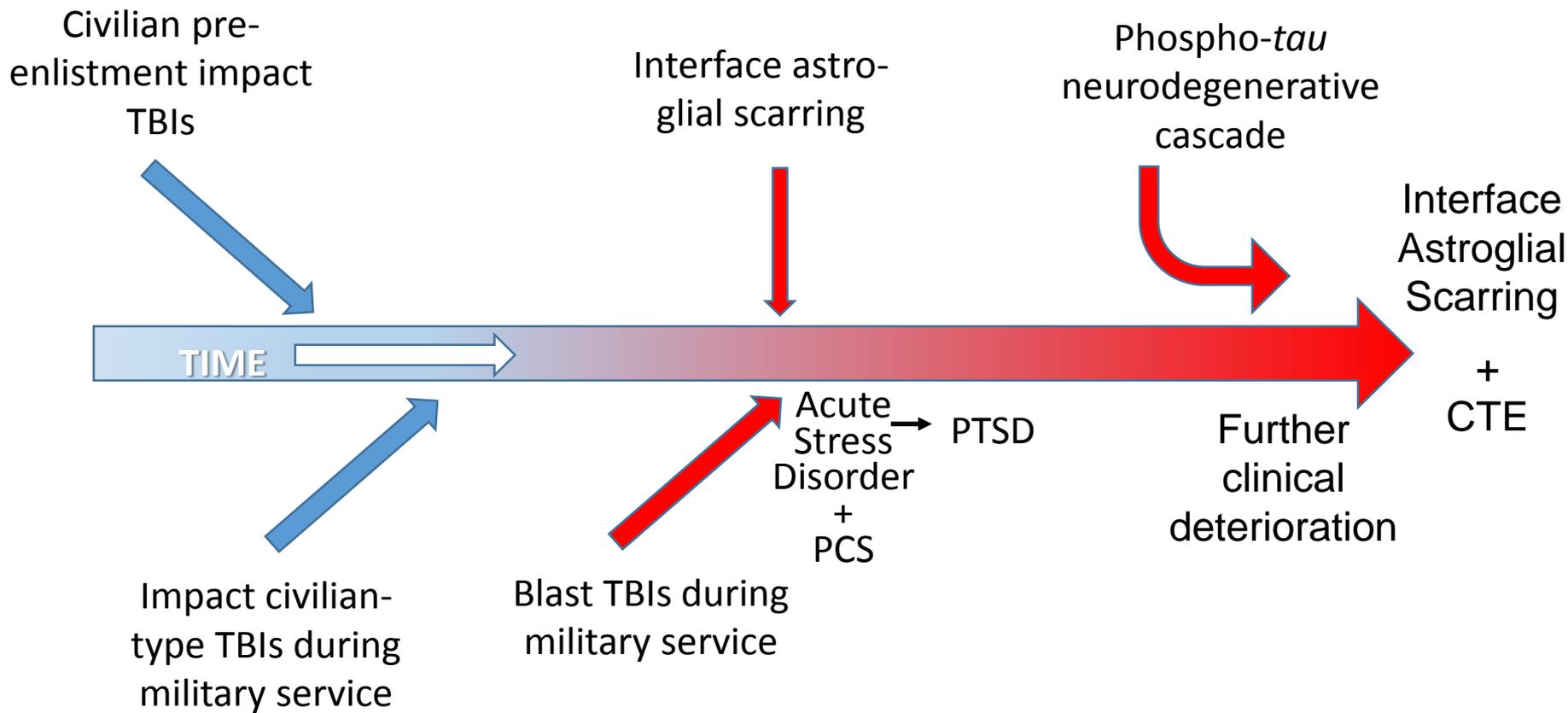
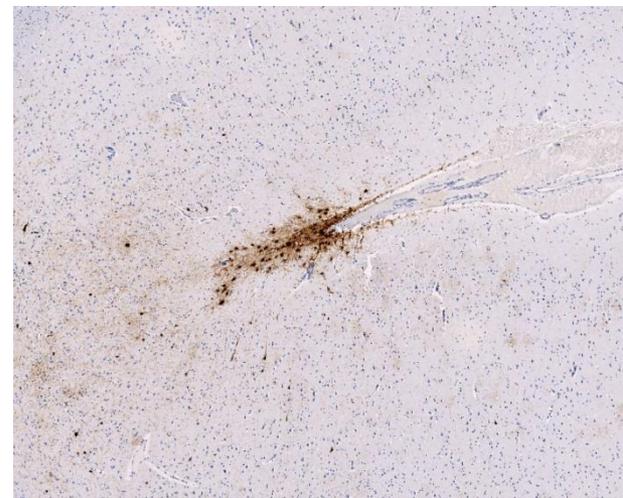
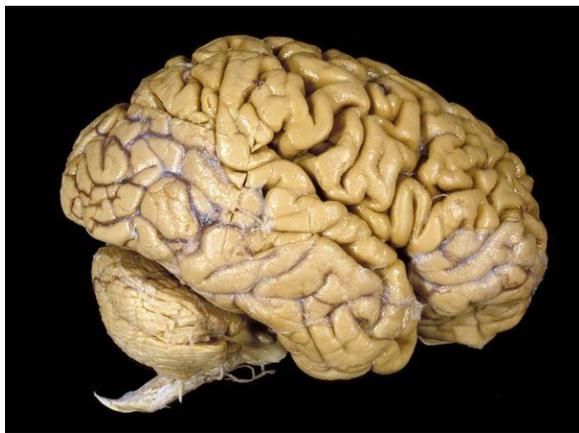
Blast TBI



Appearance of GFAP scarring on immunostained slides

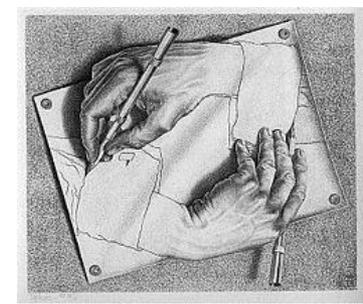
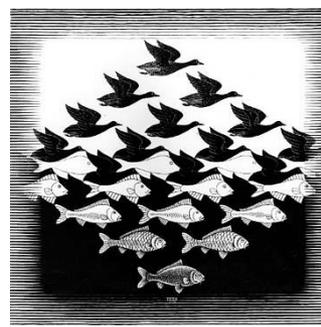


2 of the 5 chronic blast TBI cases with glial scarring also showed evidence of tau pathology (early CTE).



Implications of our findings:

- Symptomatic post-deployment Service Members diagnosed with PTSD may have distinctive microscopic brain abnormalities that cannot be detected by current brain imaging studies.
- The presence of these brain abnormalities (lesions) may contribute to the neurologic/behavioral symptoms exhibited by these patients.
- Many of the neurologic/behavioral issues of post-blast exposed service members may not be strictly related to “mental health” problems.
- Approaches to diagnosis and treatment of affected individuals should consider the potential presence of these brain lesions.



	“Organic” Disease	“Functional” Disease
	Disease of the brain	Disease of the mind
Medical Discipline	Neurologist	Psychiatrist, clinical psychologist
Underlying Abnormality	Structural abnormalities of the brain	No structural abnormalities found (perhaps “chemical imbalance”)
Examples	Stroke, Parkinson’s disease, epilepsy	Bipolar disease, obsessive compulsive disorder
Clinical Diagnostic Approach	Signs, symptoms, history, biomarkers (MRI, blood/CSF assays, EEG, etc.)	History and symptoms matching description in Diagnostic and Statistical Manual of Mental Disorders (DSM)
MRI	± abnormality seen	No abnormality seen
Therapeutic approach	Medication (L-DOPA, Dilantin), surgery (carotid endarterectomy, temporal lobe resection)	Various forms of talk therapy ± medication

Establishing the Diagnosis of Various Forms of Mental Illness – Issues With Use of the Diagnostic and Statistical Manual of Mental Disorders (DSM)

“While DSM has been described as a ‘Bible’ for the field, it is, at best, a dictionary.... The weakness is its lack of validity. Unlike our definitions of ischemic heart disease, lymphoma, or AIDS, the DSM diagnoses are based on a consensus about clusters of clinical symptoms, not any objective laboratory measure. In the rest of medicine, this would be equivalent to creating diagnostic systems based on the nature of chest pain or the quality of fever. Indeed, symptom-based diagnosis, once common in other areas of medicine, has been largely replaced in the past half century as we have understood that symptoms alone rarely indicate the best choice of treatment.” — *Thomas Insel, Former Director of the National Institute of Mental Health (as posted on NIMH Director’s blog, April 29, 2013)*

“We are hopeful that DSM-VI will be the first to incorporate biomarkers for diagnosis of at least some of the conditions contained in the new manual.” – *Stephen L. Hauser, MD and S. Claiborne Johnston, MD, PhD (Annals Neurol. 73: 5-6, 2012)*

Where do we go from here?

- Although we can only make the diagnosis of Interface Astroglial Scarring at autopsy, we can now begin to identify how common this problem is among active duty and Veteran Service members. Who develops it and who

All this developed following observations made on banked human brain specimens of what was a poorly defined condition evaluated from the two divergent viewpoints of neurologists and mental health specialists

- Develop an animal model to investigate its biology
- Prevention strategies (helmet/armor design)
- New, targeted approaches to the treatment of affected individuals

We need to pause to thank the Service members and their families who have agreed to donate the brain to our Repository.

Without this precious gift, we could not do our work.

Many of our donor families have expressed the feeling that although their loved ones have made the ultimate sacrifice, through brain donation, they continue to serve their Country. We are pleased to know that this has provided considerable comfort to them.



Acknowledgments

USUHS/CNRM/NIH

Sharon Shively
Regina Armstrong
Fabio Leonessi
Col. Geoffrey Ling (ret)
Patricia Lee
Stacey Gentile
Bernard Dardzinski
Leslie Sawyers
Diego Iacono
James Kelly
Dara Dickstein

Joint Pathology Center (JPC)

Iren Horkayne-Szakaly
Robert Jones

Harvard/MGH/Martinos Center

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Special thanks to Charles Rice, Walter Koroshetz, Steven Kaminsky, Arthur Kellermann, COL. Thomas P. Baker (ret), and MG Richard Thomas (ret)

**Funding Support from the Defense Health Program of the US
Department of Defense**

